

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
HEATH, Ellen M. *et al.*

Ref: 2902162-017000

Application No.: 10/075,593

Confirmation No.: 9392

Filed: February 2, 2002

Art Unit: 1637

For: METHOD TO ISOLATE DNA

Examiner: Strzelecka, Teresa E.

DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Dirk Loeffert, do hereby declare as follows:

1. I obtained a PhD degree in Biology from the University of Cologne, Germany in 1996. Since then, I have worked in the field of molecular biology for more than 13 years specializing in molecular biology techniques and enzymes used for modification and amplification of nucleic acids. I have worked for Qiagen GmbH since 1996. My present title is Senior Director Research North America and Senior Director R&D Modification / Amplification.
2. I am familiar with the above-identified patent application ("the present application"). I am also familiar with the Laitinen and Fairman references [collectively "the cited prior art"] that have been relied upon by the USPTO.
3. The method of the present application relates to Qiagen's PUREGENE DNA purification product, methods and kits, in particular, in connection with the Enhanced Protocol. One important advantage of a method of the present application is the fact that the biological material is contacted FIRST with a hypertonic high salt reagent to form a

suspension and THEN that suspension is lysed. This is completely the opposite of what is done in the cited prior art. Indeed, I was completely surprised that by reversing the order of these steps in the cited prior art, such an unexpected benefit of improved resuspension of cells and a therefore better accessibility of cells for lysis could be obtained.

4. I have prepared a side-by-side comparison of a method of the present application vs. Laitinen below:

| Present Application | Laitinen |
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| <p>(a) separating the biological material from the remainder of the biological sample;</p> <p>b) contacting the separated biological material comprising DNA of step (a) with a hypertonic, high salt reagent so as to form a suspension of said biological material containing DNA;</p> <p>c) contacting the suspension of step (b) with a lysis reagent so as to lyse the biological material containing DNA to form a lysate comprising DNA and non-DNA biological components released from the biological material, wherein the hypertonic, high salt reagent in step (b) comprises salt in an amount effective to precipitate proteins out of the lysate; and</p> | <p>Wash cells in PBS (pre-lysis, swelling)</p> <p>Lyse cells in RBS</p> <p>Pellet cell nuclei and resuspend in RBS</p> <p>(Remove RNA with RNase A)</p> <p>c) Lyse nuclei in lysis buffer with Prot K, incubate at 37°C</p> <p>b) Add salt for protein precipitation, vortex</p> <p>Pellet and transfer supernatant into EtOH for DNA precipitation</p> <p>Pellet, discard and supernatant</p> <p>Dry DNA pellet</p> |

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| (b) separating the DNA from the non-DNA biological components in the lysate of step (c) to yield isolated DNA. | Re-dissolve DNA pellet in TE |
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5. I have prepared a side-by-side comparison of a method of the present application with Fairman

| Present Application | Fairman |
|---|---|
| <p>a) separating the biological material from the remainder of the biological sample;</p> <p>b) contacting the separated biological material comprising DNA of step (a) with a hypertonic, high salt reagent so as to form a suspension of said biological material containing DNA;</p> <p>c) contacting the suspension of step (b) with a lysis reagent so as to lyse the biological material containing DNA to form a lysate comprising DNA and non-DNA biological components released from the biological material, wherein the hypertonic, high salt reagent in step (b) comprises salt in an amount effective to precipitate proteins out of the lysate; and</p> <p>d) separating the DNA from the non-DNA biological components in the lysate of step</p> | <p>Mix blood with RBC</p> <p>Pellet WBC</p> <p>Wash WBC</p> <p>c) Lyse WBC in lysis buffer</p> <p>b) Add salt for protein precipitation</p> <p>Pellet and obtain supernatant containing DNA</p> |

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| (c) to yield isolated DNA. | |
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6. The fact that the European Patent Office has granted claims to a method of the present application is important and I have attached a copy of the granted European Patent corresponding to the present application.

7. The fact that a method of the present application has much superior results by reversing the steps of the prior art was completely unexpected to me because a main purpose of using a high salt solution is the precipitation of proteins *following* the lysis step. These surprising and superior results are, in my opinion, most likely explained by partial or complete denaturation of cell surface proteins in the presence of high salt, thereby easing the breakage of tight cell contacts providing a better starting situation for efficient cell lysis. I would not have expected this phenomenon before. Indeed, neither Laitinen nor Fairman gave any hint that these steps should be (or for that matter even could have been) reversed because protein precipitation for purification of nucleic acids is logically only required once the cells are opened up following a lysis step. To me, as one of skill in the art, I would not have expected the method of the present application to work very well because quite commonly rather hypotonic lysis solutions are used. The fact that the steps are done in the order of first treating with a hypertonic, high salt reagent and to form a suspension and then lysing is critical.

8. I understand that the USPTO has taken the position that the best method for using a hypertonic high salt solution has not been disclosed in the present application and I do not agree as one of skill in the art. I read through the present application and on pages 10-12, many different hypertonic, high salt reagents are listed. The present application mentions that preferably the reagent has a concentration of salt that is greater than 1.0M, more preferably greater than 2.0M. Suitable salts are listed as including but not limited to sodium salts, potassium salts and ammonium salts. In the Examples, QIAGEN's PUREGENE solution is used. I can confirm that the PUREGENE solution functions as used in the examples of the present application by virtue of the fact that PUREGENE is a hypertonic high salt reagent of greater than 1.0M. I further can confirm that the

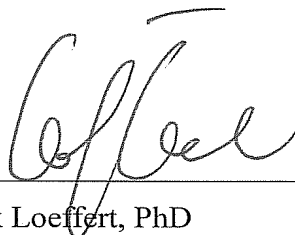
concentration of salt in PUREGENE is greater than 1.0 M and this is what makes PUREGENE function as used in the examples of the present application. It is my present belief that the other hypertonic high salt reagents greater than 1.0M listed on pages 10-12 of the present application would work just as PUREGENE works in the examples.

9. It is my opinion as one of ordinary skill in the art, that one would be able to utilize a process involving the steps I have enumerated in 4 and 5 above with many different biological samples without undue experimentation. That is, some of the sample types this process could easily be used to purify include eukaryotic cells, a physiological fluid, and/or an animal tissue.

10. It is further my opinion as one of ordinary skill in the art that obtaining a suitable hypertonic, high salt reagent that comprises salt in an amount effective to precipitate proteins out of the lysate would be something that one of skill could do without undue experimentation. This would be rather simple for a skilled person.

11. I further declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and may jeopardize the validity of the application or any patent issued thereon.

08-25-2008
Date


Dirk Loeffert, PhD